

## Prevalence of canine distemper virus in wild mustelids in the Czech Republic and a case of canine distemper in young stone martens

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**ABSTRACT:** Between 2001 and 2003, a total of 194 samples of brain tissues of wild mustelids from the Czech Republic were tested for the presence of canine distemper virus (CDV) by direct immunofluorescence examination. Out of 21 animals exhibiting symptoms of the disease or changed behaviour, one mustelid was CDV positive (5% prevalence). In this group, 1 out of 18 stone martens (*Martes foina*) was CDV positive, while 2 pine martens (*Martes martes*) and 1 Eurasian badger (*Meles meles*) were CDV negative. Of 173 animals with unknown case history, 1 sample was positive (0.6% prevalence). In this group of animals, 1 out of 19 Eurasian badgers was positive, and stone martens ( $n = 96$ ), pine martens ( $n = 4$ ), polecats (*Mustela putorius*) ( $n = 28$ ), steppe polecats (*Mustela eversmani*) ( $n = 4$ ), common weasels (*Mustela nivalis*) ( $n = 4$ ), stoats (*Mustela erminea*) ( $n = 3$ ) and American minks (*Mustela vison*) ( $n = 19$ ) were negative. Clinical distemper was demonstrated in three stone marten pup siblings. In two of the siblings, CDV was demonstrated in footpads. The third of the siblings survived the acute stage of the disease and had virus neutralizing antibodies from the end of the acute stage until 6 months after the end of the acute stage, with a maximum antibody titre of 32. During the acute stage and 7 months after the end of the acute stage, no virus neutralizing antibodies were found.

**Keywords:** genus *Morbillivirus*; direct immunofluorescence test; serology; Eurasian badger; *Martes foina*; *Meles meles*

Canine distemper virus (CDV) belongs to the genus *Morbillivirus* of the family Paramyxoviridae (Appel, 1987). Members of 8 families of the order carnivores are susceptible to the distemper virus (Montali et al., 1987), i.e. Ailuridae, Ailuropodidae, Canidae, Hyaenidae, Mustelidae, Procyonidae, Viveridae, Felidae and Phocidae (Budd, 1981; Blythe et al., 1983; Montali et al., 1983). In the order of even-toed ungulates, CDV was described in members of the family Tayassuidae (Appel et al., 1991). In primates, CDV was reported in members

of the family Cercopithecidae (Yoshokawa et al., 1989).

Distemper is an acute or subacute, highly contagious febrile disease affecting the respiratory, gastrointestinal and central nervous systems. Neurological signs may appear during the acute stage of the disease, or several weeks or even months later (Appel and Summers, 1995). Although CDV may attack animals of any age category, morbidity and mortality will differ from species to species (Budd, 1981; Appel and Summers, 1995). CDV

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produces also a chronic disease during which CDV may hide in persistently infected organs.

Distemper is considered as the most serious infectious disease in mustelids (Williams et al., 1988), and in Central Europe, in Austria and in Germany, it was reported in stone martens (*Martes foina*), polecats (*Mustela putorius*), common weasels (*Mustela nivalis*) and Eurasian badgers (*Meles meles*) (Steinhagen and Nebel, 1985; Baumgartner and Adami, 1990; Kolbl et al. 1990; Van Moll et al., 1995; Frolich et al., 2000).

In the Czech Republic, the last distemper outbreak in domestic dogs occurred between 1990 and 1993. At present, almost 70% of dogs are protected by vaccination and distemper cases in dogs are sporadic (Svoboda and Srenk, 1996). We found no reports of distemper in wild mustelids from this region of Central Europe. The aim of this study was to investigate the prevalence of CDV in wild mustelids in the Czech Republic to determine whether wild mustelids might be a CDV reservoir for animals kept by people.

## MATERIAL AND METHODS

Tissue samples of mustelids were obtained from State Veterinary Institutes in Olomouc and Prague, to where wild mustelids are sent to be tested for rabies in the Czech Republic. The samples were from either diseased mustelids or mustelids that exhibited symptoms of changed behaviour. We also received animals with unknown case history captured by workers of Institute of Vertebrate Biology of the Academy of Sciences of the Czech Republic and by hunters at different hunting grounds. In most cases, only heads or brain tissues of mustelids were sent to us. Only samples free of overt symptoms of autolysis were examined. Between January 2001 and December 2003, a total of 194 samples of brain tissue from wild mustelids were collected. Symptoms of the disease or changes in their behaviour were observed in stone martens ( $n = 18$ ), pine martens (*Martes martes*) ( $n = 2$ ) and badgers ( $n = 1$ ). The case history of other animals was unknown: stone martens ( $n = 96$ ), pine martens ( $n = 4$ ), polecats ( $n = 28$ ), steppe polecats (*Mustela eversmani*) ( $n = 4$ ), common weasels ( $n = 4$ ), stoats (*Mustela erminea*) ( $n = 3$ ), badgers ( $n = 19$ ) and American minks (*Mustela vison*) ( $n = 19$ ). The samples came from different parts of the Czech Republic, mainly from its central and eastern regions. They were col-

lected in rural areas, on the outskirts of towns, as well as in the intramural parts of towns. Samples of the cerebellum were tested for the presence of CDV by means of immunofluorescence examinations of cryosections or brain tissue smears.

In June 2003, we examined three stone marten young (two were alive, the third had died shortly before) aged about four weeks. The young were found in the attic of a cottage in the municipality of Branisov in the Sumava Mountains. The young that displayed typical symptoms of CDV infection were examined clinically. Another one died, and the two deceased young were autopsied, and histological and immunofluorescence examinations of their tissues (brain, lungs, spleen, footpads) were made. From the surviving stone marten, 5 serum samples were taken at the end of the acute disease stage, a week after the acute stage (disappearance of acute symptoms), and one, six and seven months after the acute disease stage. These serum samples were examined by the virus-neutralizing test for the presence of antibodies to CDV.

In the direct immunofluorescence examination, the commercial murine CDV FITC Conjugate (VMRD, Pullman, USA) was used. Cryosections or smears were made of frozen brain tissue samples. These preparations were left to dry and then processed by a procedure recommended by the conjugate manufacturer. A fluorescence microscope (200–400× magnification) was used for the sample evaluation.

Prior to the serologic examination, serum samples were inactivated at 60°C for 30 min. Dulbecco's minimal essential medium (DMEM) was used for serial twofold dilutions of the sera starting at the 1:2 dilution. For the examination, the Onderstepoort strain of the CDV was used (obtained from Prof. M. Vandeveld, University of Bern). The virus culture was diluted in DMEM at 100 TCID<sub>50</sub> in 50 µl. Each serum dilution was pipetted into 3 wells of a microtitration plate (50 µl per well), mixed with the virus (50 µl/well) and incubated at 37°C for 1 hour. Then 100 µl of Vero cell suspension ( $4 \times 10^4$  of cells/ml) were added to each well. The plates were incubated for 3–4 days at 37°C in 5% CO<sub>2</sub> enriched atmosphere. The antibody titre was determined as the serum dilution at which the cytopathic effect of the virus was blocked in 50% of the wells.

In the two deceased stone marten young with symptoms typical of CDV, a standard autopsy was performed. Tissue samples of brain, lungs, spleen and footpads were collected and fixed in 10% buff-

ered formalin. Paraffin blocks were made of these samples. Histological sections (5 µm) were stained with haematoxylin-eosin.

## RESULTS

### CDV prevalence in wild mustelids in the Czech Republic

Of the total of 21 examined samples from animals that exhibited symptoms of the disease or changes in their behaviour, 1 sample was CDV positive (5% prevalence). The positive sample was one out of 18 samples from stone martens (6% prevalence).

Another 173 samples from animals with unknown case history were also examined. Of those samples, one was CDV positive (0.6% prevalence). In this case, the positive sample was one out of 19 samples from Eurasian badgers (5% prevalence).

### CDV infection in three stone marten pups

Two stone marten pups were received in the acute stage of the disease, the third one had just died. Clinical examinations revealed the following symptoms: apathy, dehydration, seromucous conjunctivitis and rhinitis, haemorrhagic changes on footpads and the planum nasale, with a prominent epithelial desquamation, and watery diarrhoea. The two young ran a temperature of 39.5°C and 39.8°C, respectively. Although the young were apathetic, they showed interest in food. Re-hydration therapy was started and antibiotics were administered to both pups. One of them, however, died two days after the beginning of the treatment. The condition of the third pup improved considerably within a week. Haemorrhagic changes on footpads disappeared, but a week later papillomatous and hyperkeratotic changes (hard pad disease) began to develop on footpads of its hind feet. Neurological signs were observed in this stone marten 2–3 times a day for two weeks. While moving around, the animal suddenly fell down and remained lying prone on the floor without moving at all for about 30 seconds, and then a brief period of uncoordinated or circular movements came. The symptoms of the disease, with the exception of changes on the footpads of the hind feet, disappeared within the next week. Six months after the acute stage of the disease, similar changes began to appear also on the footpads of the front feet.

At the end of the acute stage of the disease, no neutralizing antibodies against CDV were found in this stone marten. A week after the acute disease stage, the antibody titre was 16, and in 1 and 6 months the titres were 32 and 8, respectively. No antibodies were found seven months after the acute stage.

Autopsies of the deceased animals revealed interstitial pneumonia and catarrhal enteritis. Besides the above-described changes on footpads and planum nasale, neither other pathological nor anatomical changes were found in the other organs. Histological examinations confirmed foci of interstitial pneumonia, segmental acanthosis and papillomatosis in footpads, a focus of parakeratotic hyperkeratosis of the epidermis, and sporadic vacuolization of keratinocytes in the lower *stratum spinosum*.

Direct immunofluorescence was used to examine the samples of lungs, spleen, brain and footpads for CDV. While footpad samples were positive in repeated tests, repeated tests of samples of the other tissues were negative.

All three stone marten pups were suffering from canine distemper.

## DISCUSSION

In the Czech Republic, the CDV prevalence was found in 5% of mustelids with case history of the disease or changed behaviour, and in 0.6% of mustelids with unknown case history. Prevalence of the disease in neighbouring Germany was much higher: over a period of two years, Van Moll et al. (1995) reported CDV antigens in the brains of 37% of mustelids ( $n = 146$ ) from south-western Germany sent to be tested for rabies (50 of 132 stone martens, 2 of 5 polecats, 1 of 5 Eurasian badgers and 1 of 4 weasels). Some of the mustelids showed abnormal behaviour, some were found dead, and others were killed near human habitations. The higher prevalence of CDV antigens in the examined brains of animals compared with the results reported in this study may be due to the fact that Van Moll et al. (1995) did their study during an outbreak of canine distemper in mustelids in Germany. A higher CDV prevalence in Germany was recently found by Frolich et al. (2000). They examined animals that either had been found dead or had been killed by hunters, and had unknown case history; they found CDV antibodies in 2 stone martens out of 10. Using

the RT-PCR, they also diagnosed CDV in the tissues of 2 out of 6 Eurasian badgers and in 2 samples from stone martens out of 13 examined samples. It can be explained by the much higher sensitivity of RT-PCR than is that of the standard direct immunofluorescence test.

In 21 animals examined in the present study, symptoms of a rabies-like disease were observed. The main symptoms included the loss of shyness, salivation, and uncoordinated movements or cramps leading to the death of the animal. In several cases, the diseased animals had injured people. All of the animals were examined for rabies with negative results. Distemper in badgers, martens and polecats that exhibited symptoms similar to those of rabies was already described (Kolbl et al., 1990; Baumgartner and Adami, 1990).

In the present study, only the brain tissue was examined. CDV prevalence would probably have been higher if other tissues had also been examined because the canine distemper virus may not be distributed uniformly in the organism. In the three stone marten pups, for instance, where the acute stage of distemper was monitored, CDV was ascertained in the tissue of footpads only. Different frequencies of CDV findings in different tissues in dogs with distemper were reported (Haines et al., 1999).

Bronchopneumonia, particularly of cranioventral lobes, leading to nodular changes and pulmonary emphysema is a frequent finding in martens suffering from canine distemper (Geisel, 1992). The virus antigen was not detected immunohistochemically in the lungs, neither was it detected in the lungs of the genet (*Gennetta gennetta*) that died of CDV (Lopez-Pena et al., 2001). In the present study, CDV antigen in stone martens was detected only in the skin of the footpads. Proliferative changes in the planum nasale and footpads in connection with CDV are called the hard pad disease, and they are usually described as vacuolar degeneration with the occurrence of viral inclusion bodies and massive hyperkeratosis (Alldinger et al., 1993). In stone martens with distemper examined in the present study, the main findings on the footpad were acanthosis and papillomatosis with foci of hyperkeratotic epidermis, while vacuolization was only sporadic. In dogs with the CDV-caused hard pad disease Koutinas et al. (2000) and Grone et al. (2003) also observed frequent acanthoses and no or only minimum changes like vacuolization or inclusion bodies. In the present study, no viral inclusion bodies were found in any other stone marten tis-

sues suffering from CDV. Similarly, neither intranuclear nor cytoplasmatic bodies were observed in 18 mustelids (stone and pine martens and polecats) examined by Baumgartner and Adami (1990).

The present study confirmed the presence of CDV in wild mustelids in the Czech Republic. CDV that causes the disease in the mustelids is not antigenically different from the canine *Morbillivirus* (Alldinger et al., 1993). This is the reason why mustelids may serve as a canine distemper virus reservoir for animals kept by people. The risk of infection is being aggravated by the synanthropic way of life of mustelids, which is well known in stone martens and in some areas also in Eurasian badgers (Pavlacik et al., 1994). For the above reasons, the discontinuation of preventive vaccinations of domestic animals sensitive to CDV cannot be recommended.

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